Lung Tumor Resection Does Not Affect Debrisoquine Metabolism[1]

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Abstract

Some authors have reported an association of extensive metabolism of debrisoquine with increased lung cancer risk, although others have found no association. Debrisoquine metabolism is controlled by a cytochrome P-450 isozyme encoded at the *CYP2D6* locus, which is inducible by antipyrine and rifampicin. Because lung tumors may produce a variety of humoral substances, we wanted to determine whether the tumor induced debrisoquine metabolism. As part of a case-control study of lung cancer, debrisoquine metabolism was measured in patients with histologically confirmed non-small cell lung cancer before and after surgical resection with curative intent. One hundred four incident patients with pathological stage I, II, or IIIA non-small cell lung cancer took debrisoquine (10 mg) orally at 10 p.m. and collected the subsequent 8-h urine both before and after surgery. We compared the values of the metabolic ratio, which is the percentage of the dose excreted as debrisoquine to the percentage of the dose excreted as the principal metabolite. The pre- and postoperative metabolic ratios were highly correlated (Pearson correlation coefficient = 0.96), and did not differ in value significantly ($P = 0.88$). Using traditional cutpoints (metabolic ratio, 1.0 and 12.6) to categorize the three metabolic phenotypes, the preoperative and postoperative phenotypes were well correlated ($\kappa = 0.78$). These results show that the ability to metabolize debrisoquine is not induced by the presence of a primary lung tumor.

Introduction

The differential susceptibility of tobacco smokers to lung cancer has led to a search for other contributing etiological factors. Genetic predisposition characterized by interindividual variation in drug metabolism has been an area of active investigation. The debrisoquine 4-hydroxylase genetic deficiency is one of the most widely studied human drug oxidation defects (1–5). The molecular basis for this genetic defect is a mutation in the *CYP2D6* locus on chromosome 22, which results in an absent or dysfunctional P-450 isozyme (6, 7). Approximately 8–9% of caucasians are deficient in this enzyme and are considered poor metabolizers (2). The heterozygote state may be associated with an intermediate metabolizing capability, although distinction from extensive metabolizers is difficult using the MR[3] obtained by phenotyping alone (8). Recent reports indicate that this isozyme may activate mutagenic 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (9), which is found in tobacco smoke. Previous studies have reported an association of extensive metabolism of debrisoquine with increased lung cancer risk (10–12), although others have found no association (13–18). One hypothesis is that extensive metabolizers of debrisoquine have an increased risk for lung cancer because of increased activation of procarcinogens in tobacco smoke by *CYP2D6*. However, it has been speculated that the observed association may be an effect of the tumor rather than a cause (10, 11). In theory, the tumor itself, tumor products, diagnostic procedures, or therapy could increase debrisoquine metabolism, influence the amount of drug or metabolite excreted, or interfere with the laboratory assay of the excreted products. As part of a case-control study of lung cancer, we measured debrisoquine metabolism in patients with early stage non-small cell lung cancer before and after resection of the primary tumor with curative intent to determine whether a tumor-related increase in metabolism could explain the lung cancer susceptibility/debrisoquine metabolism association observed in some studies.

Subjects and Methods

Subjects. A collaborative case-control study of incident lung cancer was conducted at the National Naval Medical Center, Bethesda, MD; Albany Medical College, Albany, NY; Laval Hospital, Quebec City, Quebec, Canada; and the Toronto Group Hospitals, Toronto, Ontario, Canada from August 1988 to February 1992. The protocol was approved by the appropriate institutional review boards, and all study

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[3] The abbreviations used are: MR, metabolic ratio; GC, electron capture gas chromatography; HPLC, fluorescence detection high-performance liquid chromatography.

patients gave signed informed consent. Patients with histologically confirmed non-small cell lung cancer felt to be suitable for complete resection with curative intent were recruited to have debrisoquine metabolism determined before and after surgery. Histological diagnoses of lung cancer were confirmed by pathology review by protocol pathologists at each institution. A normal base-line blood pressure and cardiovascular examination was required for participation. Exclusions from study participation were intensive care unit treatment, inability to give informed consent, blood pressure < 90/60, quinidine therapy, severe renal disease (creatinine > 4.0 mg/dl) or severe liver disease (total bilirubin > 3 mg/dl, serum glutamic-oxaloacetic transaminase or serum glutamic-pyruvic transaminase > 200 units/l), other active malignancy diagnosed within the previous 5 years, or chemotherapy or radiotherapy for lung cancer.

Questionnaire and Medical Record Review. An in-person interview requiring approximately 45 min was administered to the patients by a trained interviewer. Data collected included sociodemographic and anthropometric characteristics, recent and remote tobacco use, personal medical history, recent caffeine and vitamin use, alcohol use, family history of cancer and lung disorders, current medications, lifetime occupational history, and residence history. Medical records were reviewed to abstract selected information, including histological diagnoses from pathology reports, results of clinical and pathological staging, findings from operative reports, medications administered and results of clinical laboratory studies.

Debrisoquine Administration. Debrisoquine (Declinax) was obtained from Hoffman LaRoche Ltd. (Mississauga, Ont., Canada) under an Investigational New Drug approval. The subjects took debrisoquine (10 mg) orally at 10 p.m. and collected the subsequent 8-h urine specimen before surgery and repeated the procedure 2–6 weeks after recovery. Patients discarded the urine that was voided immediately before taking the debrisoquine and then collected urine for the next 8 h (including the first morning voided specimen). This procedure is similar to that used in other studies (10, 11, 19). The overnight protocol was used to minimize inconvenience for patients as well as hospital staff, and has been shown to be highly correlated with the daytime method (19, 20).

Laboratory Methods. An aliquot of urine was stored at −20°C and later analyzed for measurement of the excreted debrisoquine and principal metabolite, 4-hydroxydebrisoquine. Samples were first analyzed by a modification (11, 21) of the GC method described by Idle *et al.* (22). Because reproducibility comparisons from our study showed HPLC to be more consistent than the GC method (21), all samples in the case-control study were reanalyzed by HPLC. HPLC analyses were conducted using the method of Hempenius *et al.* (23) with a Hewlett-Packard Model 1090 HPLC (Rockville, MD) equipped with an autosampler/autoinjector and a Hewlett-Packard Model 1046A fluorescence detector. The maximum sensitivity was achieved using an excitation wavelength of 194 nm while monitoring emission at 572 nm. The separation was carried out on a SPHERI-5 CYANO (5 μm, 100 × 4.6 nm) cartridge column equipped with a 1-cm CYANO guard cartridge (Applied Biosystems, Brownlee columns). The mobile phase was prepared by adding 500 μl of triethylamine (Aldrich Chemical Company, Milwaukee, WI) to approximately 850 ml of water. Phosphoric acid (Fisher Scientific) was used to adjust the pH of the solution to 3.5. HPLC-grade acetonitrile (100 ml; Burdick and Jackson, Muskegon, MI) was added and the solution was brought to 1 liter with water. This solution was applied with HPLC-grade methanol (Burdick & Jackson, Muskegon, MI) in the proportion of 90:10 at a flow rate of 1 ml/min. The resulting mobile phase composition was 81:9:10 aqueous buffer:acetonitrile:methanol. All mobile phase components were filtered and degassed through a 0.45 μm nylon filter (Alltech Associates, Inc., Deerfield, IL). The sample size injected was 25 μl. The method was shown to be linear between 25 μg/l and 20 mg/l.

Table 1 Pearson correlation coefficients of natural log debrisoquine MR determined by pre- and postoperative high performance liquid chromatography by pathological stage

Pathological stage	Total number	Pearson correlation
I	64	0.957
II	24	0.948
IIIA	16	0.979
Total	104	0.961

Statistical Methods. The MR was determined by the ratio of percentage of the dose excreted as debrisoquine to percentage of the dose excreted as the principal metabolite, 4-hydroxydebrisoquine. The debrisoquine MR determines the debrisoquine metabolic phenotype. The phenotype definitions described by Ayesh *et al.* (10) were applied to this data for comparison of phenotype classification pre- and postoperatively, with MR > 12.6 as poor metabolizers, MR 1.0–12.6 as intermediate metabolizers, and MR < 1.0 as extensive metabolizers. A paired *t* test was used to evaluate the difference between the pre- and postoperative natural logarithm of the MR (24). Correlation between pre- and postoperative phenotype groupings was measured by a κ statistic (25).

Results

One hundred eight patients with pathological stage I, II, or IIIA non-small cell lung cancer had debrisoquine metabolism determinations both before and after surgical resection. One subject with no detectable urinary drug or metabolite in his postoperative collection and three subjects who took tricyclic antidepressants, which are metabolized at least in part by *CYP2D6*, were excluded from the analysis. The majority of patients enrolled were from Quebec (75), with 15 from Bethesda Naval, 12 from the Toronto Group Hospitals, and 2 from Albany. Sixty-two percent of the patients had pathological stage I disease. Fifty-four percent had squamous cell carcinoma and 28% had adenocarcinoma histology. The patients were predominantly white (103 of 104) and 70% were male. The median age was 63 years. Determinations of debrisoquine metabolism both before and after complete surgical resection were compared for 104 patients using HPLC and GC analyses. Both the HPLC and GC methods yielded similar MRs. Thus, results are presented only for HPLC data. The preoperative determination of the MR was highly correlated with the postoperative determination overall (Pearson correlation coefficient = 0.96) with similar results observed by sex, hospital, and stage at presentation. Correlations for stages I, II, and IIIA were 0.96, 0.95, and 0.98, respectively, as shown in Table 1. The difference between pre- and postoperative paired values was not significant ($P = 0.88$). For 56 (54%) subjects, the preoperative MR

Table 2 Correlation of debrisoquine metabolic phenotypes based on traditional cutpoints applied to preoperative and postoperative MRs calculated from high performance liquid chromatography data ($\kappa = 0.78$)

	Postoperative phenotyping			
	PM[a]	IM[b]	EM[c]	Total
Preoperative phenotyping				
PM	8	0	0	8
IM	1	21	4	26
EM	0	6	64	70
Total	9	27	68	104

[a] Poor metabolizers (MR >12.6).
[b] Intermediate metabolizers (MR 1.0–12.6).
[c] Extensive metabolizers (MR <1.0).

was slightly greater than the postoperative value. Using traditional cutpoints to categorize the three metabolic phenotypes, the pre- and postoperative phenotypes were well correlated as shown in Table 2 ($\kappa = 0.78$). Discrepancies in classification occurred most frequently between extensive metabolizers and intermediate metabolizers, where 6 of the 70 (9%) preoperative extensive metabolizers were categorized as intermediate metabolizers based on postoperative phenotyping results. Only one subject shifted from the intermediate metabolizer category to the poor metabolizer category from preoperative to postoperative phenotyping.

Discussion

We evaluated the hypothesis that primary lung cancer tumors or tumor products induce debrisoquine metabolism by measuring the metabolism before and after complete surgical resection of non-small cell lung cancers in 104 patients. Similar to other cytochrome P-450 enzymes, *CYP2D6* microsomal enzymes have been found to be inducible (2). Sparteine metabolic clearance, which is closely related to debrisoquine metabolism, was increased 30% after pretreatment with rifampicin, although only in extensive metabolizers (2, 26). Both poor and extensive metabolizers were induced by phenobarbital, antipyrine, and rifampicin pretreatment as indicated by increased excretion of 6-hydroxycortisol, a nonspecific marker of microsomal enzyme induction (2). Lung neoplasms are capable of producing a variety of humoral substances (27) that theoretically could induce microsomal enzymes and thereby enhance drug metabolizing capability.

Two smaller studies (13, 14) reported no evidence of a change in debrisoquine metabolism in lung cancer cases before and after surgery. This report is the strongest demonstration to date that debrisoquine metabolism is independent of the tumor mass. We found that the debrisoquine MR in untreated early stage non-small cell lung cancer patients is not altered by complete surgical resection of the primary tumor. Although the question of whether the association between debrisoquine metabolism and lung cancer risk observed in some studies is a cause or effect of the tumor can be addressed definitively only in a prospective study, our findings do not support the hypothesis of enhanced debrisoquine metabolism by the tumor. Consequently, it is unlikely that the reported observation of an excess number of extensive metabolizers among lung cancer cases is a result of metabolism induced by the tumor or tumor-related products. The ability to metabolize debrisoquine reflects an inherited polymorphism of drug metabolism which appears to be independent of the tumor and is reasonable to examine further as a potential biomarker of lung cancer susceptibility.

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